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ANTICATARACT EFFECT OF THE ETHANOL EXTRACTS OF *PERSEA AMERICANA AND ACTINIDIA DELICIOSA* ON SODIUM SELENITE INDUCED CATARACT IN WISTAR ALBINO RATS

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ABSTRACT

The effect of ethanolic extracts of *Persea americana* and *Actinidia deliciosa* on the oxidative stress parameters of sodium selenite induced cataract in Wistar albino rats was studied. The experimental rats were grouped into 6: Group I – Normal controls, Group II – sodium selenite cataract induced animals (developed cataract), Group III – *Persea americana* extract co-treated animals, Group IV –*Actinidia deliciosa* extract co-treated animals, Group V – *Persea americana* and *Actinidia deliciosa* extract co-treated animals (animals were co- treated for 28 days with each of the plant extract after sodium selenite induction) Group VI – standard Ascorbic acid co-treated animals(animals were co- treated for 28 days after sodium selenite induction). Body weight, organ weight and insulin level of selenite induced cataract animals decreased while plant treatment prevented this loss. Increase in values of glucose, glycated haemoglobin, creatinine, urea and uric acid levels were observed in selenite induced cataract rats, while plant treated groups restored these levels. The changes in lipid profile in Group II rats were prevented by treatment. Animals with sodium selenite induced cataract showed elevated levels of lipid peroxides and conjugated dienes, while *Persea americana*, *Actinidia deliciosa* and Ascorbic acid co-treated groups showed lower levels. Enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione S transferase) activities decreased in rats with sodium selenite cataract while both the plant and standard ascorbic acid co-treated animals showed increased enzymatic antioxidant levels. Similarly, levels of non-enzymatic antioxidants such as vitamin C and total protein decreased in sodium selenite cataract rats,

while both the plant and standard ascorbic acid treated animals showed increased levels. The above results thus, signify the antioxidative and anticataract potential of both *Persea americana* and *Actinidia deliciosa*.

KEYWORDS: Selenite induced cataract, *Persea americana, Actinidia deliciosa,* Oxidative stress, Antioxidants.

INTRODUCTION

Cataract is a multifactorial optic disorder associated with various risk factors, such as malnutrition, drugs, UV light exposure, aging and diabetes are the most significant contributors. [1,2] A higher concentration of glutathione in lens plays a major role in the protection by detoxification of drugs, reduction of active oxygen species and peroxides, as well as the disulphides and free radicals. It helps to maintain the thioldisulphide status of cells, transport of amino acids by the gamma glutamyl cycle and the removal of xenobiotic electrophiles from cells.[3] Under *in vitro* conditions, using rat lens organ culture endowed with hydrogen peroxide, low micromolar levels of flavonol quercetin inhibited oxidation-induced sodium and calcium influx and loss of lens transparency.[4] Selenite induced cataract is similar to human cataract by lipid membrane vesicles formation, increased level of calcium, elevated amount of insoluble proteins, enhanced proteolysis, and decreased amount of water soluble proteins and decline in the level of GSH.[5] It induces cataracts in young rats by various mechanisms such as calpain-induced hydrolysis and precipitation of lenticular proteins. Reactive oxygen species damage the proteins and enzymes, which mediate several cellular processes, like apoptosis that leads to the development of cataract.[6]

Persea americana contains monounsaturated fatty acids, polyunsaturated fatty acids and saturated fatty acids.[7] The dietary fiber resolves constipation, reduce fat absorption, lower glycemic index and plasma insulin levels, alter colon fermentation and microbial proliferation, and reduce plasma cholesterol.[8] *Actinidia deliciosa* fruit and skin contains flavonoids and actinidain.[9] The edible seeds contain 62% alpha-linolenic acid, an omega-3 fatty acid and antioxidants.[10] It has been used in traditional folk remedy for many diseases and show potent anti-hepatotoxic, anti-pyorrheal and prevent gingival inflammation.[11]

In our present study, we have investigated the effect of the ethanolic extracts of *Persea americana* and *Actinidia deliciosa* on the oxidative stress parameters of Sodium selenite induced cataract in Wistar albino rats.

MATERIALS AND METHODS

(i) Collection and Authentication

Edible fresh materials of *Persea americana* and *Actinidia deliciosa* were procured from the super market of Chennai, Tamil Nadu, India. The fruit samples were identified and authenticated as PRAC/2010/495 for *Persea americana* and as PRAC/2010/494 for *Actinidia deliciosa* by Botanist Prof. P. Jayaraman, Director, Plant Anatomy Research Center, West Tambaram.

(ii) Chemicals

Sodium selenite, ascorbic acid and all the other chemicals and solvent used were of analytical grade.

(iii) Extraction of Fruits

The fruits were thoroughly washed to remove the peel of both *Persea americana* and *Actinidia deliciosa* and were shade dried at room temperature for 3 to 4 days and made into coarse powder. 50gm coarse powder of *Persea americana* and *Actinidia deliciosa* were homogenized in 100ml of 90% ethanol separately using waring blender. The ethanolic extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle in refrigerator until further use.

(iv) Animal study

15 to 20 days old Wistar albino male rat pups weighing 30 ± 4 gm were purchased and maintained at Saveetha University, Chennai, Tamil Nadu, India with due permission from Institutional Animal Ethics Committee (IAEC No. SU/BRULAC/RD/) 13/2014). Experimental animals were handled according to the University and Institutional legislation, regulated by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animals were maintained under standard conditions of humidity (45-55%), temperature (25±2°C) and light (12hr light/12hr dark) in their respective groups.

(v) Experimental Design

The rat pups were induced for cataract with subcutaneous injection of sodium selenite, simultaneously then treated with both ethanolic fruit extracts, standard ascorbic acid and maintained for 28 days. Animals were grouped into six of six animals each. The groups are as follows: **Group I (Control):** Received normal saline (0.3 ml /100g BW) at every 24 hrs for

28 days. **Group II (Cataract control):** sodium selenite (30 µM / Kg BW) once induced and maintained for 28 days. The Wistar albino rat pups of Group III, IV, V and VI received single dose of Sodium selenite (30 µM / Kg BW) before treatment. **Group III:** Sodium selenite induced rats simultaneously received *Persea americana* (1gm /Kg BW) at every 24 hrs for 28 days. **Group IV:** Sodium selenite induced rats simultaneously received *Actinidia deliciosa* (1gm /Kg BW) at every 24 hrs for 28 days. **Group V:** Sodium selenite induced rats simultaneously received both *Persea americana* and *Actinidia deliciosa* (Each 1gm /Kg BW) at every 24 hrs for 28 days. **Group VI (Standard):** Sodium selenite induced rats simultaneously received Ascorbic acid (1gm /Kg BW) at every 24 hrs for 28 days.

(vi) Ophthalmic examination

Cataract was confirmed by using slit lamp biomicroscope.

(vii) Collection of Blood and Organs

After the experimental period the blood was collected without EDTA for the separation of serum and was used for biochemical parameters and then the animals were killed by cervical decapitation. The vital organs such as of liver, Spleen, kidney, heart and eyes were collected, weighed and expressed in grams/ milligrams. One of each organ were excised and rinsed in ice cold 0.9% saline solution. They were blotted dry and fixed in 10% formalin and used for histopathological studies. One eye was enucleated and the lens was dissected by the posterior approach. The lens sample were maintained at -70°C until further analysis. The lens samples were homogenized with motor driven Teflon coated homogenizer in ten times their mass of ice cold 50 mM phosphate buffer (pH 7.2) and centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatant obtained was used for the analysis of enzyme activities.

(viii) Estimation of biochemical parameters

The blood samples were used for the determination of glucose[12], Glycated hemoglobin[13] and urea[14] using standard methods. Serum insulin levels were assayed using a standard Mercodia Rat Insulin ELISA enzyme immunoassay kit from Mercodia, Sweden (cat.no-10-1124-01), Total lipids[15], Total Cholesterol[16], Triglycerides[17], Apolipo protein[18], HDL[19], LDL and VLDL level were calculated by Friedewald formula, uric acid[20], Creatinine[21] were determined by standard procedure.

(ix) Oxidative stress parameters

Lens homogenate protein[22], LPO[23], Conjugated dienes[24] and ascorbic acid[25] were determined using standard protocol. Enzymatic Antioxidants such as SOD[26], Catalase[27], Gpx[28] and GST[29] were assayed using standard methods.

(x) Statistical analysis

Values are expressed as mean ± SD for all the results. Statistical analysis was done by using SPSS 16.0.1 for One- way Analysis of Variance (ANOVA) of collected data. Difference were considered significant at * $p < 0.001$, ** $p < 0.01$.

RESULTS

Cataract Incidence

Cataract was observed after 28 days of Sodium selenite administration and verified by slit-lamp biomicroscope. Sodium selenite administered rats alone showed the gradual progression of cataract.

Body Weight

Different groups of rats showing their body weight is depicted in Fig 1. Sodium selenite induced cataract animals showed significant decrease ($p<0.001$) in body weight when compared with control animals. The animals treated with ethanol extract of *Persea americana, Actinidia deliciosa* and in combination were found to be have significant increase ($p<0.001$) in body weight when compared with cataract induced animals. Though, both plants were found to be effective in maintaining the body weight of sodium selenite induced cataract animals, *Persea americana* treated animals showed slightly better results when compared with *Actinidia deliciosa* treated animals.

Organ Weight

Different groups of rats with their organ weight is shown in Table1. There was a significant ($p<0.001$) decrease in weights of vital organs such as eye, liver, kidney, spleen, heart and lens of sodium selenite induced cataract animals when compared with control animals. The ethanolic extract of *Persea americana, Actinidia deliciosa* and in combination were found to have significant increase ($p<0.001$) in the organ weights when compared with that of animals treated with sodium selenite.

Biochemical profile of Blood

Table 2 represents the changes of blood glucose, serum glycated haemoglobin and urea levels of control and experimental animals. The levels of blood glucose, serum glycated haemoglobin and urea were significantly increased ($p<0.001$) in Group II animals when compared with Group I animals. In fruit treated animals, the levels of glucose, glycated haemoglobin and urea were significantly less ($p<0.001$) when compared to Group II animals. Treatment with fruit extracts decreased the blood glucose levels of animals to a greater extent than when compared to treatment after sodium selenite treatment. The levels of glycated haemoglobin, urea and uric acid were significantly less ($p<0.001$) in Group III, Group IV, Group V and Group VI animals.

Biochemical profile of serum

The level of serum insulin significantly decreased ($p<0.001$) in Group II animals when compared with Group I animals. The levels of insulin in both fruit treated animals increased significantly ($p<0.001$). *Persea americana* in combination with *Actinidia deliciosa* was better in increasing the serum insulin level when compared to that of *Persea americana* treatment. The levels of uric acid and creatinine increased in Group II animals when compared with that of Group I, treatment with fruit and ascorbic acid (Table 3).

Lipid profile of serum

The changes in lipid profile in serum samples of control and experimental animals were represented in Fig 2. The levels of cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol significantly increased ($p<0.001$) in Group II animals, while HDL – cholesterol level decreased significantly ($p<0.001$) when compared to Group I animals. The levels of cholesterol, triglycerides, LDL – cholesterol and VLDL – cholesterol lowered significantly ($p<0.001$) in the order of Group III, Group V, Group VI and Group IV animals when compared to that of Group II animals.

Oxidative stress parameters

The levels of lipid peroxides, protein, enzymatic and non enzymatic antioxidants in the control, cataract-induced Wistar albino rat lens and cataract induced and co- treated with *Persea americana* and *Actinidia deliciosa* were determined (Fig 3).

The level of lipid peroxides and conjugated dienes in the Wistar albino rat were determined. Group II animals showed significantly elevated ($p<0.001$) levels of lipid peroxides and

conjugated dienes as compared to Group I animals. *Persea americana* treated (Group III), *Actinidia deliciosa* treated (Group IV), *Persea americana and Actinidia deliciosa* (V), Ascorbic acid treated (Group VI) animals showed significantly lower ($p<0.001$) levels of lipid peroxides and conjugated dienes when compared to that of Group II animals. *Persea americana and Actinidia deliciosa* treated animals showed near normal levels of serum lipid peroxides and conjugated dienes compared to ascorbic acid treated animals. The formation of peroxides is more in the cataract-induced system and it decreased in co- treatment with fruit extracts. Thus both the fruits were found to be more effective in reducing the oxidative stress.

The total protein content in the lens of Group II animals showed significantly decreased ($p<0.001$) levels as compared to Group I. *Persea americana* co-treated (Group III), *Actinidia deliciosa* co-treated (Group IV), *Persea americana* and *Actinidia deliciosa* co-treated (Group V), ascorbic acid co-treated (Group VI) animals showed significantly higher ($p<0.001$) levels of protein which reached near the normal when compared to that of group II animals. The levels of vitamin C and is decreased significantly ($p<0.001$) in Group II animals when compared to that of Group I animals. Its levels were increased significantly ($p<0.001$) in both fruit co- treated and in ascorbic acid treated animals as compared to Group II animals (Fig4).

The levels of enzymatic antioxidants such as Superoxide dismutase, Catalase, Glutathione peroxidase and Glutathione S-transferase in Group II animals were significantly decreased ($p<0.001$) compared to that of Group I. Whereas, Group III, Group IV, Group V and Group VI showed significantly higher ($p<0.001$) activities of enzymatic antioxidants (Table 4).

Table 1: Organ weights of experimental animals

Groups	Liver (gm)	Spleen (mg)	Kidney (gm)	Heart (mg)	Eyes (mg)	Lens (mg)
Group I	5.12 ± 0.10	54 ± 1.16	1.25 ± 0.05	52 ± 1.20	37 ± 2.70	19.0 ± 0.80
Group II	4.45 ± 0.22a*	46 ± 1.04 a*	1.15 ± 0.04 a*	38 ± 1.04 a*	23 ± 2.0 a*	10.8 ± 1.04 a*
Group III	4.71 ± 0.35 b*	49 ± 1.89 b*	1.35 ± 0.06 b*	44 ± 1.70 b**	31 ± 1.6 b*	23.0 ± 2.1 b*
Group IV	4.70 ± 0.16 c*	52 ± 1.16 c*	1.54 ± 0.04 c*	36 ± 2.50 c*	26 ± 1.4 c*	23.0 ± 2.4 c*
Group V	4.88 ± 0.24 d*	54 ± 1.83 d*	1.36 ± 0.05 d*	43 ± 3.20 d*	24 ± 1.2 d**	21.0 ± 1.9 d**
Group VI	4.86 ± 0.21 e*	52 ± 0.98 e*	1.26 ± 0.04 e*	43 ± 2.70 e*	24 ± 1.16 e**	16.0 ± 1.1 e**

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001, **p < 0.01.

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

Table 2: The levels of glucose, glycated hemoglobin and urea in blood of experimental animals

Groups	Glucose (mg/dl)	Hemoglobin (% gm)	Urea (mg/dl)
Group I	40.6 ± 0.8	5.6 ± 1.7	25.5 ± 0.6
Group II	60.0 ± 0.7 a*	5.9 ± 0.3 a*	40.5 ± 0.8 a*
Group III	45.0 ± 0.8 b*	5.4 ± 0.2 b*	26.5 ± 0.5 b*
Group IV	42.0 ± 0.5 c*	5.4 ± 0.1 c*	27.0 ± 0.5 c*
Group V	39.0 ± 1.8 d*	5.5 ± 0.2 d*	25.0 ± 0.6 d*
Group VI	43.0 ± 0.5 e*	5.6 ± 0.4 e*	28.0 ± 0.5 e*

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

Table 3: The levels of serum uric acid, creatinine and insulin of experimental animals

Groups	Uric acid (mg/dl)	Creatinine (mg/dl)	Insulin (µU/ml)
Group I	1.25 ± 0.20	0.56 ± 0.01	8.18 ± 0.01
Group II	2.40 ± 0.01 a*	1.33 ± 0.03 a*	4.03 ± 0.01 a*
Group III	1.15 ± 0.01b*	0.83 ± 0.05 b*	5.12 ± 0.01 b*
Group IV	1.18 ± 0.01 c*	0.83 ± 0.05 c*	5.11 ± 0.01 c*
Group V	1.20 ± 0.01d*	0.81 ± 0.07 d*	6.10 ± 0.01 d*
Group VI	1.21 ± 0.01e*	0.83 ± 0.03 e*	5.20 ± 0.01 e*

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001.

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

Table 4: Activities of enzymatic antioxidants in lens of experimental animals

Groups	SOD (units/min/mg protein)	CAT (µmol of H_2O_2 consumed /min/mg protein)	GPX(µmol of NADPH oxidized/min/mg protein)	GST (µmol of CDNB conjugate with GSH/min.
Group I	1.63 ± 0.01	1.57 ± 0.02	1.67 ± 0.01	1.82 ± 0.01
Group II	0.47 ± 0.11 a*	0.63 ± 0.03 a*	0.43 ± 0.04 a*	0.40 ± 0.01 a*
Group III	0.67 ± 0.07 b*	1.27 ± 0.11 b*	1.47 ± 0.01 b*	1.03 ± 001 b*
Group IV	0.99 ± 0.03 c*	1.42 ± 0.03 c*	1.57 ± 0.01 c*	1.44 ± 0.01 c*
GroupV	1.88 ± 0.04 d*	1.55 ± 0.08 *	1.57 ± 0.01 d*	1.58 ± 0.01 d*
Group VI	1.20 ± 0.07 e*	1.42 ± 0.08 e*	1.62 ± 0.04 e*	1.68 ± 0.14 e*

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision: a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

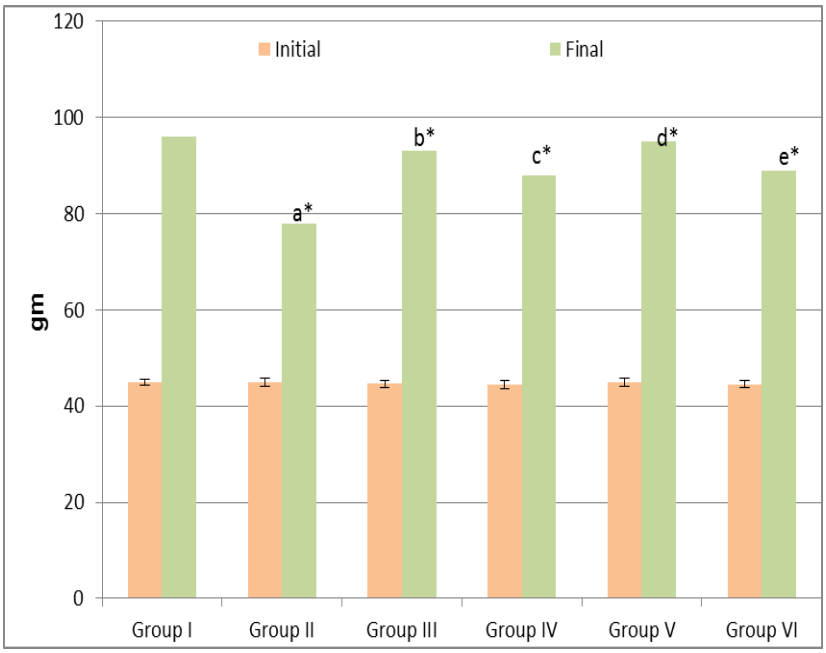

Fig 1: Body weight of experimental animals

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

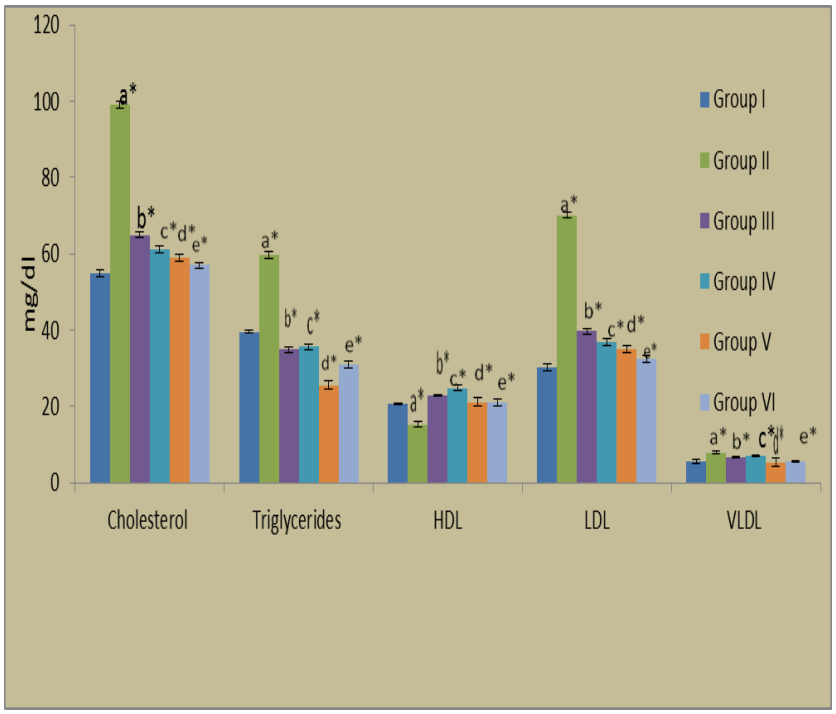

Fig2: Changes in the lipid profile in serum of experimental animals

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

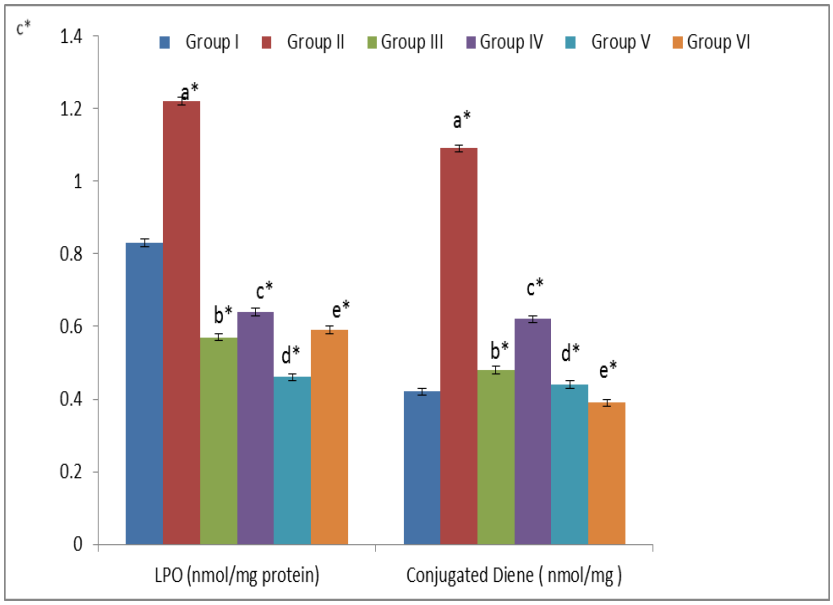

Fig3: The levels of lipid peroxides and conjugated dienes in lens of experimental animals.

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group

e- as compared with Group II

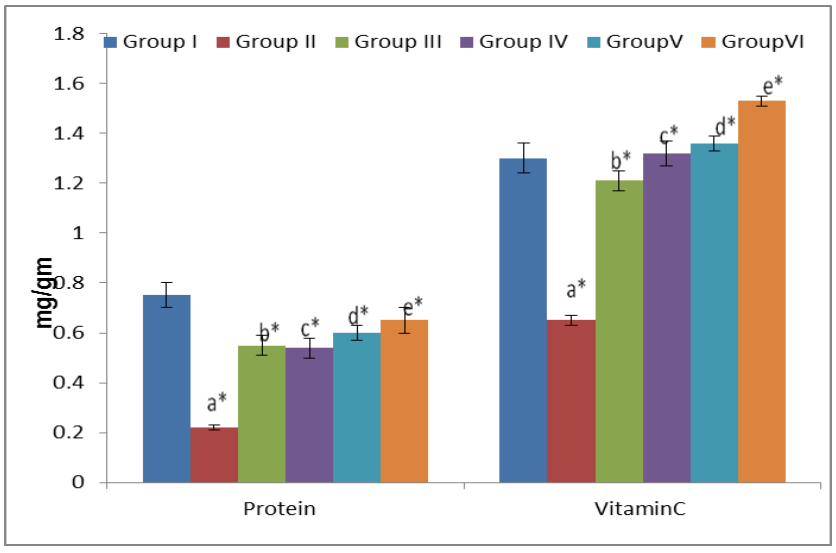

Fig 4: Levels of Protein and Vitamin C in lens of experimental animals

Values are expressed as mean ± SD for 6 Wistar albino rat in each group

Statistical significance: * p< 0.001

Comparision

a - as compared with Group I

b- as compared with Group II

c- as compared with Group II

d- as compared with Group II

e- as compared with Group II

DISCUSSION

Cataract is dependent on oxidative stress, in which oxidation of the critical sulfhydryl groups is essential for the initiation of cataractogenesis.[30] Cataract causes several biochemical processes such as altered epithelial metabolism, calcium accumulation and proteolysis, insolubilization of protein, phase transition and opacification.[31] Free radicals are capable of perturbing the homeostasis of lens leading to loss of transparency.[32] Bilateral nuclear cataract occurs within 4 to 6 days after administration of sodium selenite to the suckling rat pups before completion of the critical maturation period of the lens.[33] The flavonoid fraction isolated from fresh leaves of *Vitex negundo* protected enucleated rat eye lenses against selenite-induced cataract in an *in vitro* culture model.[34] Individuals with high plasma levels of vitamin C, vitamin E, and carotenoids appear to have reduced risk of cataract.[35] Flavonoids, phytoconstituents with antioxidant properties can prevent the oxidative damage and cataract progression.[36, 37] According to World Health Organisation about 80% of world population uses herbal medicine for health needs.[38] Thus the phytochemicals from both the fruit extract can prevent cataract formation.

CONCLUSION

Eye is a unique organ which is constantly exposed to oxidative stress, the protection of lens from these is critical for essential functioning. Although many compounds have been screened so far, no single compound has found wide spread acceptance thus, there is need for further research in this area.

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